MICROSCOPY.[1]

SOME HISTOLOGICAL METHODS BY DR. C. S. MINOT.—*Müller's Fluid.*—In hardening in Müller's fluid it is important to keep the jar with specimens and fluid in a cold place, best near freezing, for three or four days. The delicate tissues are then much better preserved; probably the same is true of Erlicki's fluid. Using Müller's fluid at a high temperature is bad for epithelia.

Beale's Carmine.—

Carmine	1 gram.
Ammonia	3 c.c.
Pure glycerine	96 c.c.
Distilled water	96 c.c.
Alcohol, 95 per cent.	24 c.c.

Dissolve in the ammonia plus part of the water; add the rest of the water and allow to stand in an open dish in a warm place until the ammonia is nearly all driven off. Then add the alcohol and glycerine. For use, dilute with an equal part of glycerine, which must be thoroughly mixed in by stirring. Stain in an open dish, which, together with a second open dish containing acetic acid, is placed under a bell glass or in a closed box. The staining requires at least twenty-four hours, and much longer if the carmine is fresh. Beale's carmine improves with use, and what is left after being employed as directed should be filtered back into the original bottle.

From the carmine solution the sections are placed in water and washed *thoroughly*, after which they are placed for 1–3 minutes in hydrochloric acid diluted with water until it tastes about like sharp vinegar. They are finally again washed in water and are then ready to mount in the usual manner.

Employed in this way Beale's carmine is one of the most valuable of histological staining fluids, both for general use and also more especially for the central nervous system. If by any chance the sections are overstained, the superfluous color may be extracted by a *brief* sojourn in *very dilute* ammonia (one drop of strong ammonia to 5 c.c. of water is quite sufficient), this is to be followed by a rapid washing in water and an immediate transfer to dilute hydrochloric acid.

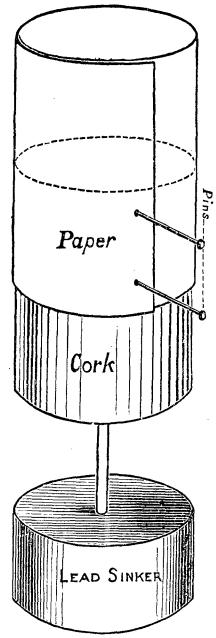
Eosine in Alcohol.—Best strength for staining is 0.0005. Dissolve one-half gramme eosine in one liter ninety-five per cent alcohol, or dissolve one gramme in 100 c.c. alcohol, and for use dilute one part of the stock solution with twenty parts alcohol. The alcoholic solution is far more convenient than the aqueous.

Imbedding in Celloidin.—The object after having been thoroughly dehydrated in alcohol is placed for twenty-four hours in a mixture of equal parts of strong alcohol and pure ether. If this mixture is kept long a little ether must be added from time to time.

[1] Edited by Dr. C. O. WHITMAN, Mus. Comp. Zool., Cambridge, Mass.

Transfer to a thin solution of celloidin, and allow it to remain for from one to three days, according to the size of the object.

Imbed in a thicker solution of celloidin. This is best done as follows: A cylindrical cork of convenient diameter is selected; a strip of glazed paper wrapped round it tightly and fastened with a couple of pins as indicated in the figure. In the box thus formed the object is placed and the celloidin poured carefully over it. If necessary the object can be secured in any position by pins. Bubbles will rise from the cork and interfere with the imbedding; two precautions will essentially diminish this danger: 1. Pour in so much celloidin that it covers the object half an inch deep, giving an opportunity for the bubbles to rise above the tissue; 2. Before imbedding cover the end of the cork with a thin layer of celloidin, which is allowed to dry on completely. After the object is covered the cork is mounted on a lead sinker (see figure), and allowed to stand until a film has formed on the upper surface. It is then immersed in alcohol of 82–85 per cent (stronger alcohol attacks the celloidin) for one to three days. I have found it best to allow plenty of time for the hardening after imbedding.



The sections have to be cut under alcohol; we use Jung's microtome with his largest knife, placed so as to cut with as much of the blade as possible; if the edge is good, then the longer the draw the thinner the sections which can be made. While cutting the knife blade should have as much alcohol upon it as possible; to secure this we use the dripping apparatus described below. The sections should be removed from the knife with a fine brush, to avoid all risk to the edge. For celloidin imbedding are needed:

1. Mixture of ether and alcohol, equal parts.
2. A thin solution of celloidin in (1). This should be syrupy but still flow easily.
3. A thick solution of celloidin in (1) of about the consistency of thick molasses.

The usual mistake is to have the solution too thick. Quantitative directions cannot be given because the celloidin varies in weight according as it is more or less dried.

Celloidin is a purified gun cotton, manufactured by E. Scherring in Berlin, Germany. It may be obtained in Boston of the Prang Educational Co., and of the Educational Supply Co., in ounce boxes, at $1.25. It is a most valuable and important addition to the resources of the histologist, as it enables him to make thin

sections of large objects; and in these sections all loose bits of tissue are kept *in situ* by the celloidin which does not interfere with the staining or mounting. If for any reason it is desired to remove the celloidin, a little ether and alcohol will dissolve it from the section almost immediately. For the study of loose parts, where the sections would otherwise fall to pieces or require difficult manipulations, such as the placenta or brain, celloidin may, I think, be safely said to surpass any other material hitherto employed.

For mounting sections with celloidin left on them I have found none of the methods hitherto recommended satisfactory. The essential oils I have tried either dissolve the celloidin like oil of cloves, or cause it to shrink and distort the section like oil of Bergamot. After trying various reagents, I have settled upon chloroform as the most convenient medium of transfer from alcohol to balsam. In using it care must be taken to place the section for half a minute in perfectly fresh alcohol, which is really 95–96 per cent; if this is done chloroform will clear it up almost immediately. When the section is in chloroform on the slide, the mounting must be expeditious, and the balsam added *while the chloroform is still covering the section.* If many sections are to be mounted, it is convenient to have a dish full of chloroform and large enough to permit plunging in the slide and placing the section on it, all under chloroform. The transfer, particularly of a large section, from the spatula to the slide, with chloroform, is often VERY difficult. To mount a single section, put it in alcohol on the slide, wash with a few drops of fresh strong alcohol; let most of the alcohol drain off, but while the section is still covered with it add chloroform, drain off the mixture, and pour over the still moist section a fresh dose of chloroform; if the washings have been really thorough the sections will clear at once.

MICROTOME KNIVES.—The Scientific Instrument Company of Cambridge, England, are preparing an automatic machine for sharpening microtome knives, which, it is to be hoped, may prove to be just what every microtomist so much needs.

It is easy enough to find men skilled in honing ordinary razors, but not so with the microtome knives. Numerous and repeated trials have been made among the most hopeful experts in and about Boston, but in no case have the knives been returned in an acceptable condition, and sometimes they have been much damaged or wholly ruined. The best test for the condition of the edge is to try it on the palm of the hand. A knife that will not cut a ribbon of paraffine sections .005^{mm} thick is not fit to use; the best knives should cut as thin as .001$^{mm.}$ It is not often that it becomes desirable to cut so thin, but it is important in making thick sections ($\frac{1}{50}$–$\frac{1}{100}$^{mm}) to use a knife that has a much finer working capacity. A thoroughly sharp blade may have very

nearly a horizontal position for its lower (plane) surface in sectioning, while a duller one requires to have its back raised a little above the level of its cutting edge. It is safe to say that a knife cuts well when thin sections (.005^{mm} or less) *agree in size with the cut surface of the paraffine block.* It may be possible to cut a straight ribbon with a dull knife, but in this case it will probably be found that the sections are shortened in a direction at right angles to the edge of the knife, which shows that the knife is acting the part of a plough, which crushes more than it cuts.

The statement that a sharp knife may have a nearly horizontal position must be understood to have some limitations. In general it may be said that the larger and harder the object the more imperative it becomes to have the under surface of the blade slant towards the object, and the necessity for this is greater with a transverse than with an oblique knife. For very hard objects a relatively thick-edged knife is required as well as a slanting position.

For ordinary histological or embryological work, the upper surface of the blade is ground hollow, the lower surface plane (Fig. 1 *k*), the edge being left very thin so that an extremely slight bevel is made in setting. What bevel there is should be mainly on the upper side. The edge when examined with a magnifying power of a hundred diameters should be perfectly straight and *smooth.*

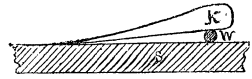


FIG. 1.—Diagram illustrating the position of the knife in sharpening. *k*, knife; *s*, oil-stone; *w*, wire.

Method of Sharpening.—Microtome knives can be properly sharpened only by those who understand their chief peculiarities, and who have trained themselves in this special work. The difficulties in acquiring the art are not, however, insurmountable; for with the proper means and a little perseverance they can be mastered in a short time. The first important step is to provide oneself either with a good razor strop (those made by Zimmermann in Berlin are considered excellent), or with a long and wide oilstone of the finest quality. Strops made of a leather band, unsupported by a solid base, and kept tense by the aid of a screw working in a frame, should never be employed in sharpening these knives, for they invariably give a bi-convex edge, with which it is impossible to do fine work. To secure a *plane* bevel of the cutting edge the surface of the strop must be perfectly *smooth, flat* and *hard.* In using the strop the knife is drawn back and forth, back foremost, *without* pressure, until the edge appears sharp when tested in the manner before mentioned.

In using an oil-stone it is well to cover the surface of the stone with a mixture of glycerine (two parts) and water (one part), as recommended by Fol.[1] The blade is laid flat on the

[1] Lehrbuch d. Vergl. Mikr. Anat., p. 129, 1884.

stone and pushed *forward, edge foremost,* in such a manner that the free end of the knife finishes by resting on the more distant end of the stone. Here the blade is turned on its back and returned, edge in advance as before, to the place of starting. In drawing the blade the utmost care should be taken *never to raise in the slightest degree the back from the stone;* and *further the knife must not be pressed on the stone, but held lightly by the finger-tips,* and the *necessary friction be left to capillary adhesion.*

After drawing the knife fifteen to twenty times it should be tested as before.

The knives furnished with the Thoma microtome should be provided with a wire support (Fig. 1 *w*) for the back of the knife during the process of sharpening.

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SCIENTIFIC NEWS.

—The results of the Hudson's Bay Expedition, in 1884, under Lieut. Gordon, possess much interest, and Dr. Bell's report on the geology of the extreme northern shores of Labrador, as well as of Hudson's strait, contains new matter especially relating to the glaciation of that country. As to the botanical collections made, Prof. Macoun affirms that it shows conclusively the arctic character of the climate of the straits and that part of Labrador north of Nachvak. Raised beaches, 300–400 feet high, were observed on Hudson's strait. Ancient stone structures, erected by the Eskimo, were observed, and Dr. Bell observes: "From what I have seen of the situations which the Eskimo in various places in Hudson's bay and strait choose for their camps, there appeared to be little doubt that they had lived here when the sea-level was 20 to 30 feet higher than it is at present."

The observations made shows that the basin of Hudson's bay may have formed a glacial reservoir, receiving streams of ice from the east, north and north-west and south and south-west. The direction of the glaciation on both sides of Hudson's strait was eastward. "That an extensive glacier passed down the strait may be inferred from the smoothed and striated character of the rocks of the lower levels, the outline of the glaciated surfaces pointing to an eastward movement, the composition of the drift, and also from the fact that the long depression of Fox's channel and the strait runs from the north-westward towards the south-east, and that this great channel or submerged valley deepens as it goes, terminating in the Atlantic ocean. Glaciers are said to exist on the shores of Fox's channel and they may send down the flat-topped icebergs which float eastward through the lower part of Hudson's strait into the Atlantic. During the drift period, the glacier of the bed of Hudson's strait was probably joined by a contribution from the ice which appears to have occupied the site of Hudson's bay, and by another also from the southward, coming down the valley of the Koksok river, and its continuation